AUTHOR(S): BUCHAN, B., KAY, G., MATTHEWS, K.H. and CAIRNS, D.

TITLE: Suppository formulations as a potential treatment for nephropathic cystinosis.

YEAR: 2012

Suppository formulations as a potential treatment for Nephropathic Cystinosis.

Barbara Buchan[a], Graeme Kay[a*], Kerr H. Matthews[a] and Donald Cairns[a].

[a]*School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, AB10 1FR, UK.*

*Corresponding author.

Dr Graeme Kay

School of Pharmacy and Life Sciences

Schoolhill

The Robert Gordon University

Aberdeen, UK

AB10 1FR

Tel: 01224 262548

Fax: 01224 262555

E-mail: g.kay@rgu.ac.uk

Abstract

Nephropathic Cystinosis is a rare autosomal recessive disease characterised by raised lysosomal levels of cystine in the cells of all organs. It is treated by the six-hourly oral administration of the aminothiol, cysteamine, which has an offensive taste and smell. In an attempt to reduce this frequency and improve the treatment, cysteamine-containing PEG suppositories were prepared and evaluated for dissolution and stability. The results demonstrated that cysteamine release was complete after 30 minutes, and that there was uniform drug distribution within the formulations. Twelve-month stability tests highlighted a potential incompatibility between some excipients, although stability was demonstrated for the cysteamine suppositories up to six months. These suppositories may provide a useful alternative to the current oral therapy for cystinosis.

Keywords: Cystinosis, Rectal Formulations, PEG.

1.0 Introduction

Nephropathic Cystinosis is a rare genetic disease characterised primarily by extremely high intracellular levels of the amino acid cystine, electrolyte imbalance, proximal renal tubular dysfunction (Fanconi syndrome) and general failure to thrive (1,2). The accumulation of cystine as crystals in most tissues leads to the progressive dysfunction of multiple organs (3). The incidence of cystinosis is one in 100,000 – 200,000 live births, and affects approximately 2000 patients in the world, although there are believed to be many more cases undiagnosed (2,4). Some infants will die due to dehydration and electrolyte imbalance from Fanconi syndrome without diagnosis (5). In the US alone there are 500-600 reported cases, with between 20 and 40 born each year (5).

Cystinosis is categorised as a Lysosomal Storage Disorder (LSD), which is a group of progressive disorders that share multi-organ failure as an endpoint (3,5). As with all LSDs, no signs of abnormality are displayed at birth; the first symptoms of glomerular dysfunction begin to appear at around 6-12 months of age (2). Without treatment most children will reach end-stage renal failure by age nine (5), and grow at 50-60% of the expected rate. By the age of eight an untreated child with cystinosis will be the height of a 4-year old (5), and, if inadequately treated, may not reach four feet in height.

The condition is caused by a defect in the CTNS gene. This is gene codes for a 367 amino-acid lysosomal transport protein called Cystinosin. In healthy cells, the amino acid cystine is transported into the cytoplasm of the cell for processing. In cystinosis however, it accumulates within the lysosomes to levels of 10-1000 times those seen in healthy cells, whereupon it crystallises from solution, causing cell death and organ failure (6). This crystallisation is widespread throughout the majority of the tissues and organs in the body (7). If left untreated symptomatic deterioration leads to death by the second decade of life (8).

The treatment for cystinosis involves the administration of the amino thiol cysteamine, which is used therapeutically in the bitartrate form as Cystagon^{TM}. With early and diligent

therapy, cystinosis patients can prevent or delay most of the non-renal complications and extend their lives into a fourth or fifth decade, live independently and some women have borne children (5,9). The renal damage is continuous however, and decline is inevitable (5).

Cysteamine causes a range of side effects, and this is largely due to the high dose which is required as much of the drug is lost to first-pass metabolism (9). High blood levels must be achieved, as a large proportion of the administered drug will bind to circulating proteins, and cannot be up taken by the cells (10,11). Patients should aim to take their dose at regular 6-hour intervals for the treatment to work effectively, as the plasma half-life of cysteamine is 1.88 hours (7), with blood levels peaking at 1h, and rapidly declining thereafter (5). This is a lifelong commitment and requires the patient to wake in the middle of the night (9). The drug has a foul taste and smell akin to rotten eggs, which regularly induces vomiting after ingestion (9). In approximately 10-15% of patients this can be severe enough to halt therapy (5). Cysteamine and its metabolites are excreted in breath and sweat, and this is also an issue, especially when the child enters education. Cysteamine has the potential to cause potentially serious stomach irritations such as gastric acid hypersecretion, reverse peristalsis and bile reflux, and 97% of patients report gastrointestinal symptoms (12,13), which in many patients is severe enough to significantly limit therapy (14,15,16). Compliance can therefore be a major barrier to effective treatment, and lead to significant morbidity.

The rectal route of administration can largely avoid the phenomenon of first-pass effect. This results from one of the three rectal veins draining into the hepatic system, while the middle and lower veins bypass this and drain directly into the systemic circulation. If the suppository is positioned correctly, the drug should not be subjected to the first pass effect. This potentially allows a smaller dose to be administered, thereby reducing or eliminating some of the unpleasant side effects. They may also be beneficial for treating conditions in infancy, when capsules are difficult to administer, or when the oral route is compromised. Rectal formulations are useful tools, particularly in a case such as this where the taste and side effects renders the task of swallowing a tablet very unpleasant and foreboding

1.1 Aims

Formulation science may provide a way to improve the current medication, significantly improving the lives of sufferers and those who care for them. By eliminating the taste and frequency of administration through alternative dosage forms, ease of administration of cysteamine could be improved. The aim of this work was to develop alternative formulations of cysteamine which could reduce or eliminate some of the side effects experienced using the current oral capsule, thereby improving the quality of life for those affected. An improvement in the ease of administration of cysteamine to infants and young children was a central objective, therefore suppositories were investigated. By avoiding the first-pass metabolism of cysteamine, a lower dose should be achievable, while the taste and upper-gastric side effects should be eliminated. A study conducted by Van't Hoff and co-workers was previously undertaken where a cysteamine-loaded suppository gel, for use in cystinosis, was evaluated. However this rectal formulation was eliminated before cysteamine absorption was completed (17).

2.0 Materials and methods

2.1 Materials

Cysteamine hydrochloride and polyethylene glycol grades 400, 600, 1000, 1500, 3000, 4000, 6000, 8000 and 14000 were obtained from Sigma. Witepsol W35 was obtained from Gattefosse (St-Priest, France). Gelucire 39/01 was purchased from Sasol GmbH (Witten, Germany). Poloxamer F68 was bought from BASF SE (Ludwigshafen, Germany). Ellman's Reagent, 5,5'-dithiobis(2-nitrobenzoate) (DTNB) was purchased from Molekula (Gillingham, UK). Tris buffer and Tween 80 were bought from Fisher.

2.2 Synthesis of N, N-(Bis-L-phenylalanyl)cystamine bistrifluoroacetate, (Phenylalanine conjugate).

Cysteamine does not possess a chromophore and therefore is UV transparent, thus monitoring its release from formulations is very difficult. Initially, a phenylalanine conjugate was developed to tag the molecule, allowing quantitative determination of release of the active from the dosage form via UV spectroscopy (figure 1). Cystamine, the oxidized disulphide of cysteamine was used in the synthesis. The phenylalanine conjugate was subsequently replaced with cysteamine hydrochloride with DTNB detection (see dissolution studies).

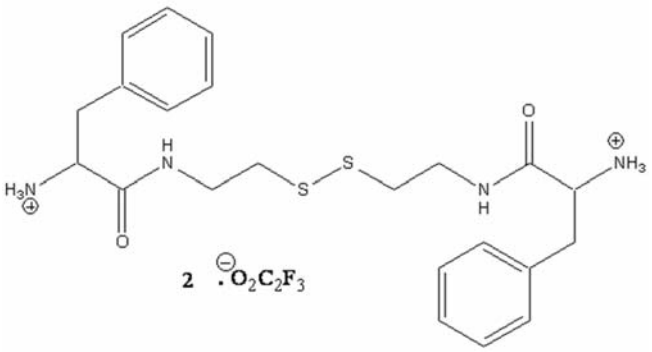

Figure 1: N, N-(Bis-L-phenylalanyl)cystamine bistrifluoroacetate, (Phenylalanine conjugate)

To a stirring solution of cystamine dihydrochloride (1g, 0.00444 moles) in anhydrous dichloromethane (20 cm^3) at room temperature, 1,8-diazabicycloundec-7-ene (1.33 ml, 0.0089 moles) was added. The reaction mixture was then stirred at room temperature for 1 hour. To this was added butoxycarbonyl-L-phenylalanineN-hydroxysuccinimide ester (3.22 g, 0.0089 moles). After thin layer chromatographic analysis, the reaction mixture was then partitioned between dichloromethane (20 cm^3) and water (50 cm^3). The dichloromethane extracts were washed with water (3 x 50 cm^3) and dried over magnesium sulphate. The resultant solution was applied to a silica gel chromatography column (4 x 30 cm^3) prepared with dichloromethane. The column was initially eluted with 100% DCM

followed by 2% graduations of methanol, with the protected compound eluting at 9:1 DCM : methanol.

The protected compound was dissolved in trifluoroacetic acid (5 cm^3) at room temperature. After 3.5 hours, the trifluoroacetic acid was removed by co-evaporation with ethanol (3 x 20mls). Trituration with diethyl ether followed by filtration and drying (vacuum oven at 50°C) gave the target compound as an off – white solid. Yield: 42%.

Found:

Mass spectroscopy: m/z 447.2 (100%); 224.4 (50%). M: $C_{22}H_{32}N_4O_2S_{22}^+$; Exact Mass: 448.2.

NMR: All analyses were undertaken using a Bruker Topspin Ultrashield 400MHz (Massachusetts, USA). ^{1}HNMR spectrum (CD_3OD) (400MHz) δ: 2.55 (m, C*H*$_2$-Ar, 2H); 2.95 (m, CH*H*-S_2, 1H); 3.05 (m, C*H*H-S_2, 1H); 3.25 (m, α-C*H*, 1H); 3.3 (m, NH-C*H*H, 1H); 3.45 (m, NH-CH*H*, 1H); 3.95 (t, N*H*, 1H); 7.25 (m, Ar-*H*'s, 5H). ^{13}CNMR spectrum (CD_3OD) (400MHz) δ: 37.7(*C*H_2-S_2); 38.8 (β-*C*H_2); 39.5 (NH-*C*H_2); 55.8 (α-*C*H); 128.9 (Ar-*C*H); 130.1 (Ar-*C*H); 130.5 (Ar-*C*H); 135.6 (Ar-*C*H); 169.7 (*C*=O). Melting range: 100-104°C

2.3 Suppository preformulation and manufacture

To allow for wastage, the weight of eight suppositories was calculated for a six-form mould. The calibration value was calculated for each mould, and the displacement value was calculated for each drug. The bases were weighed accurately on a Mettler AE50 analytical balance, along with the active. The bases were heated and blended together, before addition of either cysteamine or phenylalanine conjugate. This mix was then thoroughly stirred and the resultant liquid poured into a mould and allowed to cool. The tops were then trimmed with a spatula, and the final forms removed from the mould, bottled and labeled (BP, 2005). The surfactant Tween 80 was used in some formulations to ensure the fatty bases were sampled accurately from the dissolution medium.

Lipophilic bases, hydrophilic bases and blends of different PEG grades were examined for optimum characteristics. As 10 mg $kg^{-1}$was used for previous rectal delivery of cysteamine, 60mg was used as a nominal value (17). Until further tests are carried out *in vivo*, the correct dose cannot be determined.

Suppository hardness was tested on an Erweka TBH28 hardness tester (Heusenstamm, Germany). A hardness value of at least 1.8-2kg is considered acceptable for unmedicated suppositories (18). If the inert suppository blend produced unsatisfactory results beyond this range, no further testing was performed. The suppositories with the best characteristics, based on hardness tests and appearance were selected for further testing.

2.4 Dissolution studies

Dissolution tests were performed in a six-chambered Sotax CH-4123 dissolution bath (Basel, Switzerland). The dissolution test method followed the standard BP test for suppositories: one suppository in a stainless-steel basket, rotating at 100 rpm in a 1L beaker containing medium at 37°C, sampling at 5 minute intervals for 1 hour (BP 2005 Edition). The dissolution medium was altered depending on the active used. The release was measured by UV spectroscopy. The samples were returned to the bath after analysis. Suppositories containing the phenylalanine conjugate were initially tested in distilled water, using UV absorbance at 256 nm corresponding to the λmax.

Cysteamine hydrochloride release can be measured via free sulphydryl concentration through the use of DTNB (Ellman's Reagent, 5-(3-Carboxy-4-nitrophenyl)disulphanyl-2-nitrobenzoic acid). Basically, one mole of DTNB reacts with one mole of cysteamine to release one mole of 2-nitro-5-thiobenzoate (NTB). The concentration of NTB and hence the concentration of cysteamine can be determined using UV-Vis spectroscopy. Calibration measurements, using an analytical wavelength of 440 nm, gave a linear response, R^2=0.9991. The dissolution medium was 90% deionised water and 10% 1M Tris buffer solution at pH8. Due to the susceptibility of DTNB to photolysis when in solution, the tank was surrounded by aluminum foil and protected from light.

The Higuchi equation was used to determine the rate-order of the drug release. A result below 0.45 is indicative of Fickian diffusion (19).

2.5 Active dispersion studies

In a suppository, there is a possibility that the tip might contain more drug than the rest of the form due to gravitational settling (18). To ensure uniform drug loading, three separate areas of the suppository were sampled and compared using a DSC Q100 Differential Scanning Calorimeter (TA instruments Delaware, USA) and the experiment carried out in triplicate. Coupled with this, DSC studies of suppositories were carried out, to determine the thermal characteristics of each suppository blend.

2.6 Stability tests

Stability studies were conducted over a 12-month period. Upon manufacture (T_0), the suppositories were stored under three different environmental conditions in order to determine their stability; a refrigerator at 4°C and 8 %RH (Relative Humidity), a store containing a desiccator with saturated sodium hydrogen sulphate solution at 20°C and 52 %RH (20), and an Environmental Test Chamber (Copely, Nottingham, England) set at 30°C and 75 %RH (21). These conditions were selected to simulate cold storage, typical home storage and accelerated testing. As the suppositories had low melting points, it was decided to reduce the commonly used accelerated temperature from 37°C to 30°C. The temperature in each of the storage areas was monitored daily. DSC and IR spectroscopy were undertaken every week for one month, every month for 3 months, and subsequently every 3 months for 12 months, and compared with the T_0 results (21). IR Spectroscopy, was undertaken using a Nicolet IS10 IR from Thermo Scientific (Fisher, UK) with a Smart iTR attachment and a diamond HATR (horizontal attenuated total reflectance). Thiol groups absorb at a wavenumber of 2600 to 2550cm^{-1}, while disulphides absorb between

620 and 600cm^{-1}(22). These measurements were used as the basis for the comparisons made between samples.

Samples of uniformly less than 10mg were placed in a standard aluminium pan for DSC analysis. The DSC was set to a heat-cool-heat cycle, where the sample was equilibrated at 0°C, then heated to 100°C at 10°C per minute, equilibrated at 100°C, then cooled at 10°C per minute to 0°C, before being heated again to 100°C at 10°C per minute.

3.0 Results and discussion

The final three suppositories selected for further characterisation were blend "A" (40% PEG 8000, 60% PEG 600), blend "B" (40% PEG 14000, 60% PEG 600) and blend "C" (PEG 1500) (table 1). There is a large variation in release onset in forms A, B and C, which is illustrated by large standard deviation values. T_{100} for PEG blends A, B and C were observed at 20, 45 and 60 minutes respectively (n = 5-9). PEG blend C released the phenylalanine conjugate more slowly than PEG blends A and B. This may be due to the greater hardness of blend C (table 2).

Table 1. A summary of the batches made and their characteristics.

Suppository batch	Composition (% w/w)	Hardness without active	Hardness including active (Cysteamine HCl)	Appearance
A	PEG 8000 40% PEG 600 60%	19N, 1.94 kg	9N, 0.92 kg	Uniform white
B	PEG 14000 40% PEG 600 60%	17N, 1.73 kg	12N, 1.22 kg	Uniform white
C	PEG 1500 100%	30N, 3.06 kg	27N, 2.75 kg	Opaque

Table 2. Summary of phenylalanine conjugate release studies

Percentage release	Time to release phenylalanine conjugate (minutes, ± SD)				
	PEG blend A	PEG blend B	PEG blend C	Gelucire	Witepsol
10%	2 (± 7.1)	2 (± 6.3)	3 (± 5.1)	0.5	0.5
25%	5	5	9.5	1	1
50%	7	7.5	12.5	2	2
75%	10 (± 5.6)	10 (± 5.4)	17 (± 8.6)	2.5	2.5
100%	20 (± 0.6)	45 (± 0.8)	59 (± 0.7)	4.5	4.5

The fatty bases Witepsol and Gelucire produced a more homogenous blend and melted instantly (data not shown), whereas the PEG bases produced a more prolonged dissolution profile and dissolved more slowly over time (figure 2). It was decided to continue studies using PEG bases only, as they produced a more prolonged release of 20-60 minutes compared to 2 minutes for Witepsol and Gelucire. This slower dissolution of the PEGs allows the drug to be in contact with the rectum for longer where it is more likely to be absorbed into the body. A sudden melt over 2 minutes would increase the chances of the drug being lost through expulsion.

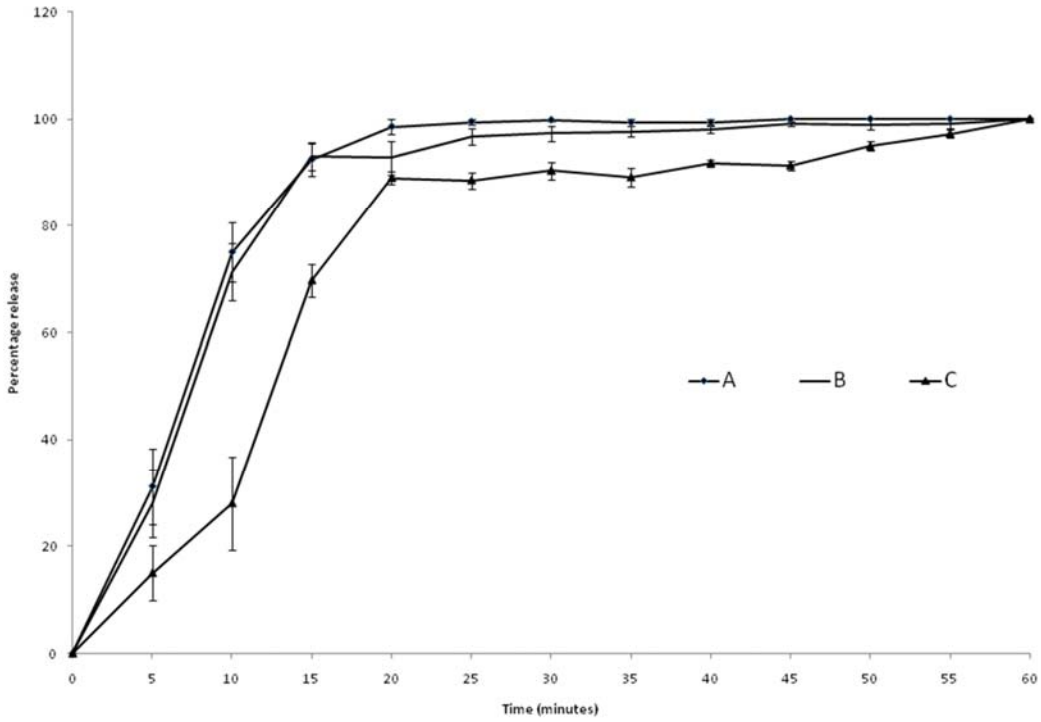

Figure 2. Percentage release of the phenylalanine conjugate from PEG Blends A, B and C over time
(n = 7, 9 and 6 respectively)

Figure 3 illustrates the release of the active from the three suppository bases over time. Cysteamine hydrochloride was used as the active, formulated into suppository bases PEG blends A, B and C.

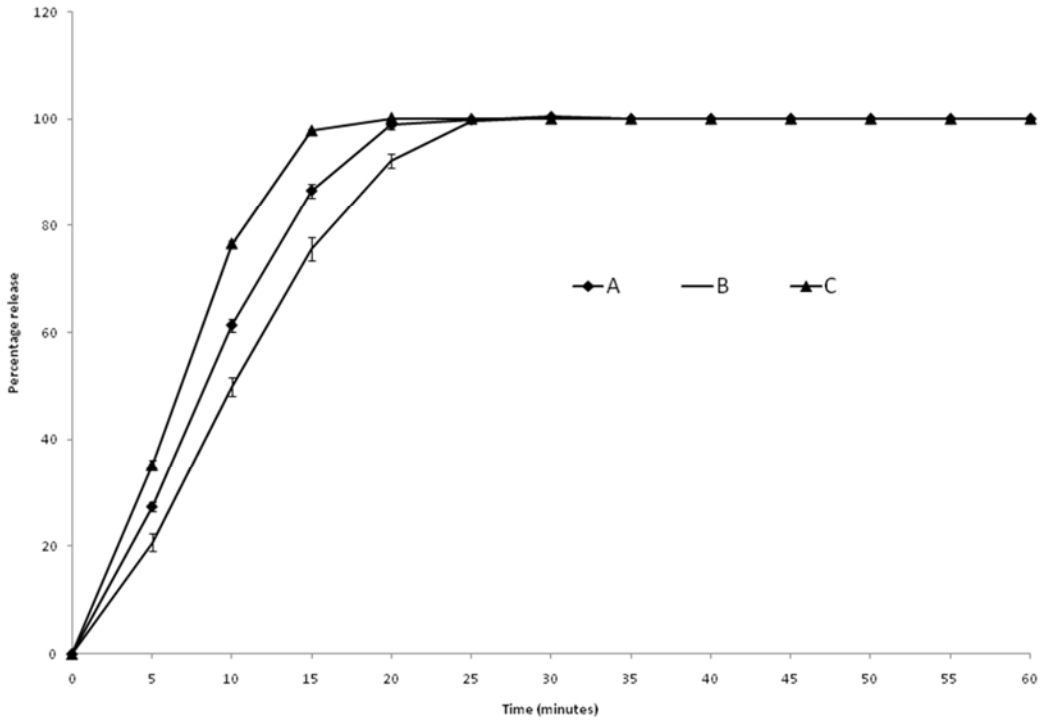

Figure 3. Percentage release of cysteamine from PEG Blends A, B and C over time (n = 6, 5 and 5 respectively).

All three PEG blends containing cysteamine hydrochloride as the active displayed more reproducible profiles than dissolution using the phenylalanine conjugate. The PEG bases should dissolve at the same rate in every replicate, and this may have been due to non-uniform drug loading of the phenylalanine conjugate. The phenylalanine conjugate was developed initially to allow release from the dosage forms, to be monitored spectrophotometrically. It should be noted that cysteamine hydrochloride is a difficult drug to formulate. At room temperature it undergoes oxidation to a disulphide form, cystamine, which itself has been shown to deplete cells of cystine but which is not licensed for use (5,23,24). Cysteamine is also extremely hygroscopic and deliquescent. However, the introduction of DTNB as a quantitative reagent allowed measurement of cysteamine directly. A summary of the release times obtained is shown in table 3. The data produced using Ellman's reagent demonstrated excellent reproducibility (average standard error of the mean for PEG Blend: A – 0.39, B – 0.58, C – 0.14).

Table 3. Summary of cysteamine hydrochloride release studies

Percentage release	Time to release cysteamine hydrochloride (minutes)		
	PEG blend A	PEG blend B	PEG blend C
10%	1.75	2.5	1.75
25%	5	6.5	4.5
50%	9	10	7
75%	13	15	9.5
100%	26	26	17

3.1 Active dispersion studies

To ensure cysteamine hydrochloride was evenly dispersed throughout the suppository, samples were taken from three separate portions of the suppository and analysed using DSC (figure 4). There was minimal difference between the tip, edge and middle sections of the suppository suggesting a uniform dispersion of active within the PEG suppository.

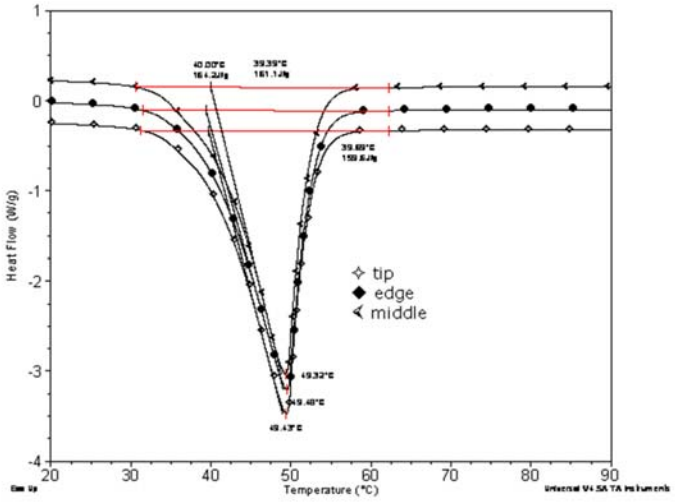

Figure 4. Section comparison of the melt phase between the tip, middle and edge areas of the PEG Blend A suppository containing cysteamine hydrochloride.

3.2 Stability tests

The temperature and relative humidity in each of the three storage chambers were monitored continuously throughout a 12 month period. The average results obtained are shown in table 4.

Table 4. Average temperatures and relative humidities in each storage chamber, with standard deviations (n = 3).

	Refrigerator	Store	ETC*
Average temperature	3.7°C (± 0.8)	20.3°C (± 1.9)	29.9°C (± 1.0)
Average relative humidity	9.2% (± 0.3)	52%	76.4% (± 2.0)

*Environmental Test Chamber

3.3 DSC results: T_0, T_6 months, T_{12} months comparison.

The thermal properties of the suppositories were assessed at time zero (T_0), six (T_6) and twelve (T_{12}) months storage at 4°C/8 %RH, 21°C/52 %RH and 30°C/75 %RH. The main melting endotherm of PEG in the broad range of 30-60°C was analysed in terms of the onset of the melt (T_{onset}), the peak temperature (T_{max}) and the total enthalpy (W/g). Selected data are presented in table 5 as an increase or decrease in temperature and enthalpy compared with samples at T_0. An exemplary thermogram of PEG blend C suppositories at T_0, T_6 and T_{12} is also included (figure 5).

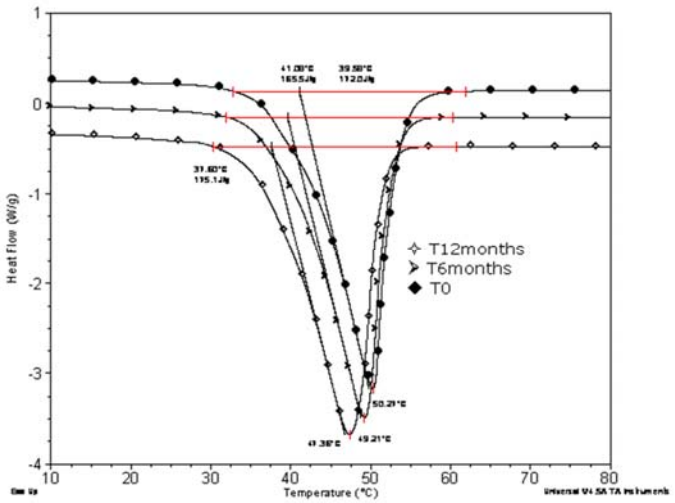

Figure 5. An Exemplary thermogram of PEG blend C supositories.

Reference to the data in table 5 indicates significant changes to T_{onset} and T_{max} for aged samples of PEG blend A at 30°C, blend B at 21°C and blend C at both 21 and 30°C. Suppositories stored at 4°C demonstrated less certain variation in T_{onset} and T_{max} as may have been expected for samples stored at this refrigerated temperature. Although the bulk of the temperature variations were considered to be within the error of measurement for DSC analyses, it is clear that changes to the degree of crystallinity at the higher storage temperatures had occurred. A depletion in the crystalline character of PEG suppositories may be expected based on lengthy exposure to high humidity and temperature, exacerbated by the hygroscopic (deliquescent) nature of contained cysteamine hydrochloride. There is some evidence of increased crystallinity for PEG blend B stored at 21°C, considered as a 'lamellar thickening' of ordered ethylene oxide units (25), but the overall data set suggests a decrease in the molecular order of cysteamine containing PEG suppositories at elevated temperatures over time. Increased enthalpies for the melt transition in all samples are a consequence of a broadened endotherm, symptomatic of increased disorder in crystalline domains rather than increased order which would produce clear increases to values of T_{onset} and T_{max} and a sharpening of the melting event. Such changes are not evident.

Table 5. DSC analysis data of selected suppository samples over time (n = 3).

Suppository sample	Time zero T_{onset} (°C) ΔH (J/g) T_{max} (°C)	T_{6} months T_{onset} (°C) ΔH (J/g) T_{max} (°C)	T_{12} months T_{onset} (°C) ΔH (J/g) T_{max} (°C)
PEG blend A	50.6	+ 0.4	- 0.9
4°C storage	71.8	+ 4.2	+ 9.4
	56.4	+ 0.3	- 1.3
PEG blend A	50.7	+ 1.1	- 0.2
21°C storage	83.8	+ 9.0	+ 13.3
	57.7	+ 0.4	- 0.5
PEG blend A	50.5	- 1.9	- 5.9
30°C storage	75.3	+ 8.8	+ 14.5
	56.6	- 0.8	- 5.1
PEG blend B	51.4	+ 0.8	- 0.3
4°C storage	73.4	+ 7.3	+ 9.1
	57.8	- 0.1	- 1.3
PEG blend B	50.4	+ 2.3	+ 0.63
21°C storage	67.2	+ 19.2	+ 22.8
	56.7	+ 1.7	+ 0.2
PEG blend B	50.7	+ 0.9	- 0.8
30°C storage	80.1	+ 6.1	+ 10.9
	57.6	- 0.5	- 1.7
PEG blend C	41.2	+ 1.6	+ 0.9
4°C storage	159.4	+ 20.4	+ 26.5
	50.3	- 0.3	- 0.2
PEG blend C	41.1	- 1.5	- 3.5
21°C storage	165.9	+ 6.5	+ 9.0
	50.3	- 1.1	- 2.9
PEG blend C	50.0	- 0.7	- 15.3
30°C storage	164.5	+ 6.1	- 8.1
	40.9	- 2.5	+ 5.3

3.4 Infrared Spectroscopy after 1 week, 3 months, 6 months and 12 months.

Infrared spectroscopy utilizes a set of unique peaks on a spectrum which can be used for identifying a compound. Each of the suppository samples was determined by IR spectroscopy and added to a compound library. A percentage match was then performed on each sample (table 6).

The oxidation of cysteamine to cystamine was the basis of comparisons made between samples. This reaction produces a peak in the region of disulphide bond stretch, i.e. 620-600 cm^{-1}, and allows a simple identification of sample degradation.

Each suppository was analysed over time using the IR spectrometer, and compared to the suppositories at time zero. The results are shown in table 6 (selected examples only).
The stability tests using IR analysis demonstrated that PEG blend C was the most stable in all conditions, with minimal changes over six months at room temperature and accelerated tests, and up to twelve months stability when stored at 4° C. PEG blend B shows evidence of stability over twelve months at room temperature. PEG blend A shows evidence of degradation when subjected to a range of storage conditions, and therefore is unsuitable for the rectal delivery of cysteamine.

Table 6. Suppository stability over time, presented as percentage match to sample at T_0 (selected data only).

Suppository blend/storage conditions	Percentage match to sample at T_0
PEG blend C, 4°C/8% RH	99.2% at 3 months
	98.84% at 12 months
PEG blend A, 4°C/8% RH	97.4% at 3 weeks
PEG blend C, 21°C/52% RH	98.94% at 6 months
PEG blend B, 21°C/52% RH	97.95% at 12 months
PEG blend C, 30C/75% RH	98.94% at 6 months

3.5 Correlation between DSC plots and IR percentage match results

The IR percentage match data support the DSC plots. This is evidence of correlation between the two methods and allows a more conclusive result to be produced. For example, PEG blend C stored at 21°C displayed a 98.94% match at 6 months (table 6).

These stability test results support the hardness testing, and indicate that PEG Blend C was the most stable formulation over a 12-month period, and indicate that PEG Blend C was the most stable formulation over a 12-month period. The suppositories stored at 4°C were generally more stable than those stored at higher temperatures, although PEG blend C displays long term stability even at room temperature. PEG blend B suppositories were stable over time at room temperature. PEG blend A suppositories displayed long term instability under all storage conditions. PEG blend C displays ideal stability over time in a range of storage conditions and is proposed as the optimal formulation in this study.

4.0 Conclusions

Liquid gel suppositories containing cysteamine have previously been investigated for the treatment of cystinosis (17). However, due to the osmotic nature of the suppository vehicle, the doses were rapidly expelled and cysteamine blood plasma concentrations were insufficient to produce a reduction in mean leukocyte cystine concentration. In this project, the suppository bases Witepsol, Gelucire and PEG were investigated for their suitability as vehicles for the incorporation of cysteamine hydrochloride and a phenylalanine-cystamine conjugate. Melting point, hardness, stability and appearance were analysed, and the three suppository bases with the most suitable characteristics were chosen for further study. PEG blends A, B and C were made and characterised. Blend C displayed good qualities (i.e. complete, reproducible release after 30 minutes, stability over 12 months at 4°C/8% RH) required for the delivery of cysteamine hydrochloride to the rectum. DSC analysis indicated that cysteamine hydrochloride was dispersed uniformly in the suppositories. Dissolution studies using the phenylalanine-cystamine conjugate revealed 20-60 minute release, while cysteamine hydrochloride as the active component demonstrated 17-26 minute release on average. The stability tests indicate that 4°C/8% RH provided the ideal storage conditions over a 12-month period. Formulation C was the most stable over time, while blend A was the least stable form, and even when stored in the refrigerator was subject to degradation. There was also evidence of an incompatibility between cysteamine hydrochloride and PEG Blends A and B, and this may be a combination of physical ageing

of the PEG and syneresis of cysteamine (26-29). There was evidence that crystal ripening over time is forcing the expulsion of the cysteamine hydrochloride to the outside surfaces of the suppository. The highly deliquescent cysteamine then quickly dissolves in environmental moisture, forming droplets of liquid on the surface of the suppositories. For these reasons, PEG blends A and B would not be suitable for the rectal delivery of cysteamine. Analysis indicates that PEG blend C would be an ideal suppository base for the delivery of cysteamine hydrochloride.

These tests demonstrate that cysteamine hydrochloride can be formulated as a suppository, and that the bases can tolerate varying amounts of drug loading. This will be of particular benefit when treating cystinosis during infancy, and should allow a significant reduction in side effects, improving compliance and morbidity. In addition, suppositories may help to eliminate the overnight treatment break which is difficult to overcome with the oral capsules. Future work with the suppositories will involve the development of *in-situ* gelling forms, and should include *in-vivo* testing or an *in-vitro in-vivo* correlation (IVIVC) to investigate the potential benefits these forms may have compared to the current oral treatment.

Acknowledgements

The authors gratefully acknowledge financial support from the Cystinosis Foundation, UK and TENOVUS, Scotland.

References

(1) Almond PS, Matas AJ, Nakhleh RE, Morel P, Troppmann C, Najarian et al. Renal Transplantation for Infantile Cystinosis: Long-Term Follow-Up. *Journal of Pediatric Surgery.* 1993; 28(2):232-238.

(2) Gahl WA, Kleta R. Nephropathic Cystinosis in Adults: Natural History and Effects of Oral Cysteamine Therapy. *Annals of Internal Medicine.* 2007; 147(4):242.

(3) Syres K, Harrison F, Tadlock M, Jester JV, Simpson J, Roy S, et al. Successful treatment of the murine model of cystinosis using bone marrow cell transplantation. *Blood.* 2009; 114(12):2542-2552.

(4) Cystinosis Research Network. *About Cystinosis.* [homepage on the Internet]. Illinois, USA: Cystinosis Research Network; 2007 cited 2007 20/5/07]. Available from: www.cystinosis.org/symptoms-treatments.html.

(5) Gahl WA. Cystinosis. *Pediatric Nephrology.* 2009; 6:1019-1038.

(6) Thoene JG. A Review of the Role of Enhanced Apoptosis in the Pathophysiology of Cystinosis. *Molecular Genetics and Metabolism.* 2007; 92 (4): 292-298.

(7) Kleta R, Gahl WA. Pharmacological treatment of nephropathic cystinosis with cysteamine. *Expert Opinion on Pharmacotherapy.* 2004; 5(11): 2255-2262.

(8) Antignac C, Hippert C, Dubois G, Morin C, Disson O, Ibanes S, et al. Gene Transfer May Be Preventative But Not Curative for a Lysosomal Transport Disorder. *Molecular Therapy.* 2008; 16(8):1372-1381.

(9) Dohil R, Fidler M, Gangoiti JA, Kaskel F, Schneider JA, Barshop BA. Twice-daily cysteamine bitartrate therapy for children with cystinosis. *The Journal of Pediatrics.* 2010; 156(1):71-75.

(10) Schneider JA, Belldina EB, Huang MY, Brundage RC, Tracy TS. Steady-State Pharmacokinetics and Pharmacodynamics of Cysteamine Bitartrate in Pediatric Nephropathic Cystinosis Patients. *British Journal of Clinical Pharmacology.* 2003; 56:520-525.

(11) Schneider JA, Fidler MC, Barshop BA, Deutsch R, Martin M, Dohil R et al. Pharmacokinetics of Cysteamine Bitartrate Following Gastrointestinal Infusion. *British Journal of Clinical Pharmacology.* 2006; 63(1):36-40.

(12) Wenner WJ, Murphy JL. The Effects of Cysteamine on the upper Gastrointestinal Tract of Children with Cystinosis. *Pediatric Nephrology.* 1997; 11(5):600-603.

(13) Schneider JA, Dohil R, Newbury RO, Sellers ZM, Deutsch R. The Evaluation and Treatment of Gastrointestinal Disease in Children with Cystinosis Receiving Cysteamine. *Journal of Pediatrics.* 2003; 143(2):224-230.

(14) Bendel-Stenzel MR, Steinke J, Dohil R, Kim Y. Intravenous delivery of cysteamine for the treatment of cystinosis: association with hepatotoxicity. *Pediatric Nephrology.* 2008; 23:311-315.

(15) Lim J, Pellois JP, Simanek EE. A retro-inverso TAT-like peptide designed to deliver cysteamine to cells. *Bioorganic & Medicinal Chemistry Letters.* 2010; 20 (21): 6321-6323.

(16) Walker WA. The stomach and duodenum. In: Walker WA, editor. *Pediatric gastrointestinal disease: pathophysiology, diagnosis, management, Volume 1.* Fourth ed. USA: PMPH-USA; 2004. p. 516.

(17) van't Hoff W, Baker T, Dalton RN, Duke LC, Smith SP Chantler C et al. Effects of Oral Phosphocysteamine and Rectal Cyseamine in Cystinosis. *Archives of Disease in Childhood.* 1991; 66:1434-1437.

(18) Allen LV. *Suppositories.* first edition ed. 2008: Pharmaceutical Press; 2008.

(19) Peppas NA, Sahlin JJ. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *International Journal of Pharmaceutics.* 1989; 57:169-172.

(20) O'Brien FEM. The Control of Humidity by Saturated Salt Solutions. *Journal of Scientific Instruments.* 1948; 25:73-76.

(21) Aulton M. E. *Pharmaceutics. The Science of Dosage Form Design.* 2nd ed. London: Churchill Livingstone; 2002.

(22) Coates J. Interpretation of Infrared Spectra, A Practical Approach. In: Meyers RA, editor. *Encyclopedia of Analytical Chemistry.* Chichester: John Wiley and Sons Ltd; 2000. p. 10815-10837.

(23) Schneider JA, Thoene JG, Oshima RG, Crawhall JC, Olson DL. Cystinosis. Intracellular Cystine Depletion by Aminothiols In Vitro and In Vivo. *Journal of Clinical Investigation.* 1976; 58:180-189.

(24) Skovby F, Kleta R, Anikster Y, Christensen R, Gahl WA. Cystinosis in Denmark: Mutation analysis and treatment with cystamine, the disulphide of cysteamine. *American Journal of Human Genetics.* 2002; 71(4): 413.

(25) Marand H, Huang Z. Isothermal lamellar thickening in linear polyethylene: Correlation between the evolution of the degree of crystalling and the melting temperature. *Macromolecules.* 2004; 37:6492-6497.

(26) Zobrist B, Weers U, Koop T. Ice nucleation in aqueous solutions of polyethylene glycol with different molar mass. *Journal of Chemical Physics.* 2003; 118(22):10254-10261.

(27) Li Y, Kaito A. Highly oriented structure formed in a lamella-forming diblock copolymer with high molar mass. *European Polymer Journal.* 2006; 42:1986-1993.

(28) Talibuddin S, Runt J, Liu L, Chu B. Microstructure development and crystallization of Poly(ethylene oxide) and melt-miscible PEO blends. *Macromolecules.* 1998; 31:1627-1634.

(29) Hay J. The physical ageing of amorphous and crystalline polymers. *Pure and Applied Chemistry.* 1995; 67(11): 1855-1858. It is available from: http://iupac.org/publications/pac/pdf/1995/pdf/6711x1855.pdf